

Detection of CYP3A1 in Formalin-Fixed, Paraffin-Embedded Rat Tissue

Reagent and Antibody Information

[1X Wash Buffer](#)

[3% Hydrogen Peroxide](#)

[1% BSA Diluent](#)

[1X Citrate Buffer](#)

[DAB Chromogen](#)

[Hematoxylin](#)

Blocking Solution: Dakocytomation Protein Block Serum-Free Ready-To-Use

Dakocytomation Corporation

Carpinteria, CA 93013

www.dako.com

1-800-235-5763

Code No. X0909

Avidin / Biotin Blocking Kit

Vector Laboratories, Inc.

Burlingame, CA 94010

www.vectorlabs.com

1-800-227-6666

Catalog # SP-2001

Primary Antibody: Rabbit Anti-Rat Cytochrome P450 Enzyme CYP3A1 Antibody

Millipore

Billerica, Massachusetts 01821

www.millipore.com

1-800-645-5476

Catalog # AB1253

Negative Control Serum: Normal Rabbit Serum

Jackson ImmunoResearch Laboratories, Inc.

West Grove, PA 19390

www.jacksonimmuno.com

1-800-367-5296

Catalog # 011-000-001

Staining Kit: LSAB+ System-HRP

Dakocytomation Corporation

Carpinteria, CA 93013

www.dako.com

1-800-235-5763

Code No. K0690

Note: This kit includes reagents needed for the secondary antibody (link) and label complex.

Staining Procedure

Positive Control Tissue: Liver (upregulated by treatment)

Stain Localization: Cytoplasmic

1. Deparaffinize and hydrate slides through the following solutions:

Solution	Repetitions	Time
Xylene	2 times	5 minutes
100% Ethanol	2 times	3 minutes
95% Ethanol	2 times	3 minutes
1X Wash Buffer	2 times	5 minutes

2. Quench endogenous peroxidase by placing the slides in 3% hydrogen peroxide for 15 minutes.

3. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.

4. Heat-Induced Epitope Retrieval Using The Steamer

Add distilled water to the bottom portion of the steamer.

Preheat 200ml of 1X citrate buffer in a Tissue Tek® container in the steamer between 95°C and 100°C.

Immerse a full rack of slides into the citrate buffer and place the lid back on the steamer.

(Insert blank slides into any empty slots in the rack to ensure even heating of slides)

Steam the slides for 20 minutes.

Remove container from steamer and cool for 20 minutes.

Rinse the slides in 2 changes of distilled water for 3 minutes each time.

5. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each time.

6. Block with the Dako protein-blocking reagent for 10 minutes at room temperature.

Lot # _____ Exp. Date _____

DO NOT RINSE THE SLIDES. CONTINUE TO AVIDIN-BIOTIN BLOCK.

7. Avidin / Biotin Blocking Kit

Lot # _____ Exp. Date _____ New Kit: yes / no

Apply avidin block for 15 minutes at room temperature.

Quick rinse in 1X wash buffer.

Apply biotin block for 15 minutes at room temperature.

DO NOT RINSE SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.

ONLY WIPE EXCESS BLOCK.

8. Apply the primary antibody at a 1:1500 dilution. Incubate for 30 minutes at room temperature.

Lot # _____ Exp. Date _____

For negative control slides, dilute normal rabbit serum so that its protein concentration matches that of the primary antibody (if necessary). Then make a 1:1500 dilution. Apply the negative and incubate for 30 minutes at room temperature.

Lot # _____ Date Reconstituted _____

9. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.

LSAB+ Kit

Lot # _____ Exp. Date _____

10. Apply the Link (yellow bottle) from the LSAB+ Kit. Incubate for 30 minutes at room temperature.

11. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.

12. Apply the Label (red bottle) from the LSAB+ Kit. Incubate for 30 minutes at room temperature.

13. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.

14. Apply the DAB chromogen. Incubate in the dark for 6 minutes at room temperature.
(Add 1 drop of DAB per ml of substrate)

Lot # _____ Exp. Date _____ New Kit: yes / no

15. Rinse the slides in tap water 3 minutes.

16. Counterstain with hematoxylin for 20 seconds.

17. Rinse the slides in tap water until water is clear.

18. Gently agitate slides in 1X wash buffer until the tissues turn blue.

19. Dehydrate through the following solutions:

Solutions	Repetitions	Time
95% Ethanol	1 time	3 minutes
100% Ethanol	3 times	3 minutes
Xylene	2 times	5 minutes

20. Coverslip

Updated 04/28/11